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Role of specific antibodies in *Coxiella burnetii* infection of macrophages

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Q fever is a zoonosis with worldwide distribution caused by *Coxiella burnetii*, an obligate intracellular bacterium. Q fever is commonly divided into acute and chronic forms. Acute Q fever manifestations consist of self-limited febrile illness, pneumonia and granulomatous hepatitis as well as neurological disorders and miscellaneous manifestations [1]. The manifestations of chronic Q fever are endocarditis and, less frequently, vascular aneurysm and prosthesis infections. These usually occur in patients with previous vascular or valvular disease or in a context of immunosuppression. The chronic disease is characterised by impaired immune response, defective microbicidal activity of monocytes, and production of antibodies (Abs) directed against *C. burnetii*. Here, we examined the role of specific antibodies in the infection of human macrophages by *C. burnetii*.

Immunoglobulins G (IgG) from three patients with chronic Q fever and three healthy controls were purified using protein A-sepharose columns (Pharmacia, Uppsala, Sweden). Their ability to opsonise *C. burnetii* was investigated by flow cytometry. About 85% of phase I organisms were opsonised by specific Abs (1/200 dilution) whereas irrelevant IgG were unable to opsonise bacteria. The intracellular fate of opsonised organisms within human monocyte-derived macrophages (MDM) was studied by real-time PCR as previously described [2]. Opsonised organisms intensively replicated within MDM whereas unopsonised organisms survived but poorly replicated: after 9 days, the bacterial load was 10 times higher in MDM infected with opsonised bacteria (98 400 vs. 10 200 *C. burnetii* DNA copies for 10^5 MDM). In addition, electron microscopy revealed that the replication of opsonised organisms was associated with the formation of spacious parasitophore vacuoles containing large amounts of bacteria (Fig. 1a), which were absent in MDM infected with unopsonised bacteria (Fig. 1b). At day 9 post-infection, about 30% of MDM infected with opsonised bacteria presented large vacuoles (Fig. 1c) whereas only 3% of MDM displayed large vacuoles when infected with unopsonised bacteria (Fig. 1c). Note that the large parasitophore vacuoles presented an electron-dense membrane and seemed unable to fuse with lysosomes (Fig. 1a). This prompted us to characterise the nature of the large vacuoles by scanning fluorescence confocal microscopy using cathepsin D as a marker of phagolysosomes [3]. It has been previously shown that *C. burnetii* prevents the phagosome conversion and survives in monocytes/macrophages within late phagosomes, in contrast to avirulent and inactivated organisms, which are eliminated within phagolysosomes [3,4]. At day 9 post-infection, opsonised organisms did not co-localise with cathepsin D (Fig. 1d), demonstrating that the replicative large vacuoles are not phagolysosomes.

Classically, it is believed that Abs provide protection against pathogens through different mechanisms, including opsonisation. We demonstrated here that the high levels of specific Abs found in chronic Q fever, which are likely to be related to the host inability to cure the infection, favoured *C. burnetii* replication within human MDM. In patients with *C. burnetii* endocarditis, the major manifestation of chronic Q fever, *C. burnetii* was found in infected cardiac valves as voluminous intracytoplasmic masses within infected mononuclear cells, and not extracellularly as for other aetiological agents of infective endocarditis [1]. We can hypothesise that *C. burnetii*-specific Abs produced during chronic Q fever are responsible for *C. burnetii* infection of cardiac valves. We also demonstrated that *C. burnetii* prevented the phagosome conversion because large parasitophore vacuoles containing organisms did not express cathepsin D, a specific

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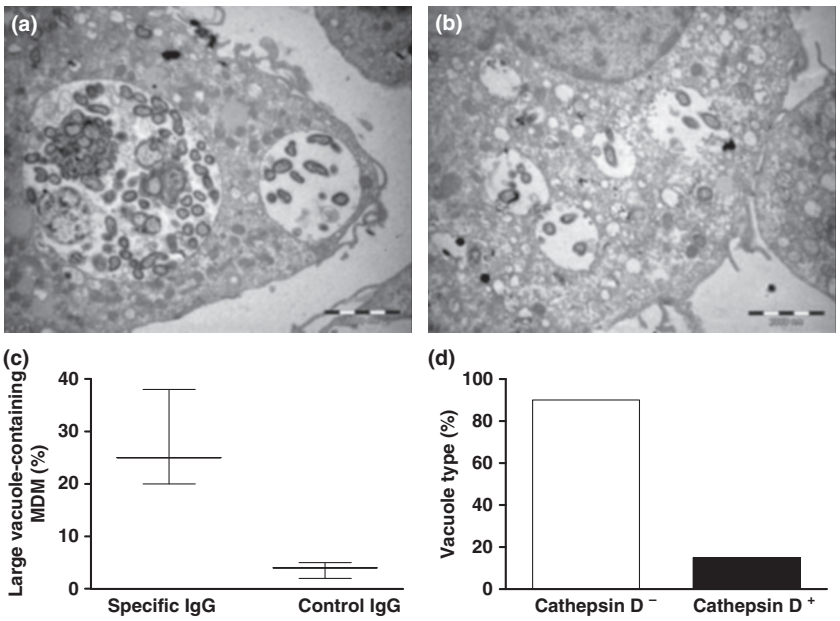

Fig. 1. Formation of large replicative vacuoles in MDM. Purified IgG from patients with chronic Q fever or healthy subjects were incubated with *Coxiella burnetii* for 2 h. MDM (10^5 cells/well) were infected with opsonised (a) and unopsonised (b) *Coxiella burnetii* (bacterium-to-cell ratio of 200:1) for 4 h. MDM were then washed to remove free bacteria and incubated for 9 days before electron microscopy examination. The percentage of MDM containing large vacuoles was determined (c) and the proportion of vacuoles that fuse with lysosomes (cathepsin D^+ compartment) was determined by fluorescence scanning confocal microscopy (d).

marker of phagolysosomes. Similar results have been described during *Leishmania* infection, where IgG are not protective and induce the replication of parasites within large vacuoles [5]. Taken together, our results reveal an unexpected and detrimental role for Abs in the chronic evolution of an infectious disease.

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